Ventricular Nonmixing as a Source of Error in the Estimation of Ventricular Volume by the Indicator-Dilution Technic

By H. J. C. Swan, M.B., M.R.C.P., Ph.D., and Walter Beck, M.D.

Bing and co-workers described a method for the measurement of volume, based on the washout of material following its injection into the right ventricle, by sampling the blood-indicator mixture from the pulmonary artery with a long catheter. Distortion in the estimate of the rate of clearance of dye from the pulmonary artery was corrected for by an arbitrary calibration. Holt applied the same principle with improved techniques and estimated stroke and residual volumes for the left ventricle. The variability and the magnitude of the residual volume of the left ventricle (1.1 to 4.7 ml./kg. of body weight) reported by Holt for the intact dog anesthetized with morphine, allobarbital (Dial) and pentobarbital were somewhat unexpected in relation to the values for pulmonary artery-to-aorta “transit-time volumes” reported from this laboratory.

We have repeated Holt’s experiments using densitometer-catheter systems that are apparently capable of recording close to the true concentration of indicator in the aortic root at the end of diastole. The magnitudes of volume so determined agree in general with those of Holt and also demonstrate considerable variability. Evidence is presented to demonstrate that newly entering left atrial blood does not mix completely with the contents of the ventricle. Since, therefore, the ventricle may not behave as a one-chamber emptying system, such calculations of volume may be subject to large errors.

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Methods

Theoretic Considerations

The underlying concept in basic treatment has been clearly presented by Holt and will be briefly summarized here. The symbols used by Holt are adhered to throughout.

\[
Q (C_1 - C_m) \over C_1 = S = \frac{EDV - ESV}{1 - k} (1)
\]

\[
S = \frac{EDV}{ESV} (2)
\]

In the equations, \( S \) signifies stroke volume; \( Q \), quantity of indicator injected; \( C_1 \), \( C_2 \), etc., concentrations of indicator immediately downstream to the ventricle in which injection has been made; \( EDV \) and \( ESV \), end-diastolic and end-systolic volumes respectively; and \( k \), average of the ratios of successive concentration values, or

\[
k = \frac{C_s/C_1 + C_s/C_2 + \ldots + C_s/C_{m-1}}{m - 1} (4)
\]

The assumptions stated or implied by Holt include: (1) complete mixing of the blood and injected dye in the ventricle; (2) constancy of \( ESV \), \( EDV \) and \( S \) for the few seconds of the determination, (3) concentration recorded in the aorta during diastole as equal to the concentration in the ventricle during the immediately preceding systole, and (4) absence of significant reflux of dye into the left atrium.

Material

Studies are reported on 11 dogs anesthetized with morphine sulfate and chloralose (5.0 ml. of 1 per cent solution of chloralose per kilogram of body weight).

For the injection of dye a no. 6 standard-bore catheter was introduced into the left ventricle either by a pretracheal technic or by a transternal septal technic shown us by Dr. David Donald. For the injection of indicator the tip of the catheter was occluded and radially 0.5 to 1.5 cm. from the tip. The catheter was filled with dye and injection was accomplished by air pressure (20 to 31 p.s.i.). The volume delivered and the timing of the
Aorta

**Figure 1**

Response of catheter-densitometer systems used for recording of aortic-root concentration curves to sudden changes in dye concentration. In the left panel the true change in concentration of blood entering the catheter is shown by the lower line indicating the position of a stopcock which determines whether blood with or without dye enters the catheter-densitometer system; the upper curve shows the response of the system. Data from a series of such determinations are plotted in the right panel, in which the time for the instrument to attain 90, 80 and 70 per cent of the final (6 seconds) concentration value was plotted against the flow rate through the system.

Injection were regulated by a solenoid valve actuated by a variable delay circuit triggered from the electrocardiogram. The volumes delivered and the duration were indicated on the record and were approximately 1.0 to 1.5 ml. and 0.16 to 0.26 second respectively. A 40-cm. no. 6 L catheter was placed in the ascending aorta above the aortic valve via the right common carotid artery for sampling of the blood-dye mixture. For some experiments, sampling was also carried out from the femoral artery or from a second site in the ascending aorta. In each instance the sampling catheter was 40 cm. long.

The concentration of the blood-dye mixture drawn through the sampling catheter was recorded by means of the cuvette densitometer of high dynamic response designed by Sutterer and Wood. The responses of such catheter-densitometer systems are greatly influenced by the flow rate through them. The response to a square wave input of dye for the aortic sampling system is shown in figure 1. The effect of flow on the time required to attain 90, 80 and 70 per cent of the final concentration for both the aortic and the pulmonary system is plotted in figure 1. The severe damping effect of slow sampling rates on these systems is readily apparent. Particular attention was therefore paid to the attainment of the highest possible flow rates for these sampling systems and the recording of such flow rates. The former objective was accomplished by application of negative pressure (-21 p.s.i.) to the top of a calibrated buret. The rate of ascent of blood in this system was recorded in milliliters per second. The dilution curves, respiration, femoral-artery-pressure, electrocardiogram and other variables were recorded photographically. In some early experiments, recording was done with a Heiland "visicorder."

In another group of experiments, cineangiograms were recorded simultaneously with indicator-dilution curves. For this purpose, 5 to 8 ml. of 70 per cent solution of acetrizoate (Urokon) was injected (1.2 to 2.0 ml./sec.) via the catheter in the left ventricle. Cardiogreen was added to the acetrizoate to give final concentrations of 0.2 mg./ml. or an injected dose of 1.0 to 1.6 mg. The cineangiograms were made with a Westinghouse cinefluoroscope at 30 frames per second.

**Results**

An example showing the dilution curve recorded at the aortic root following injection into the left ventricle is given in figure 2. The heart rate was 135 beats per minute. This curve demonstrates satisfactory resolution of dye concentrations attained with a higher-
Dilation curve recorded from the aortic root following injection of indicator into the left ventricle (dog 4). In the left panel an original roentgenogram is reproduced showing placement of a catheter in the left ventricle (indicated by the arrow) by the pretracheal technic. A second catheter, also indicated by an arrow, records the concentration of dye in the aorta, 1.5 cm. above the aortic valve. Catheters A and B are positioned in the right ventricle and left pulmonary artery respectively for similar determinations relative to the right ventricle. The central panel shows a dilution curve recorded at the aortic root. Note the occurrence of an extrasystole coincident with the injection of indicator which in this instance occupied 0.15 second. After a pause of approximately 0.8 second the dilution curve is inscribed and is characterized by a steplike decline in concentration from its peak. In the right panel, note the logarithmic decline in end-diastolic concentration with successive ejections. Below this panel are the calculated data for the curve shown.

Determination of Left Ventricular Volumes

The values determined for stroke and end-diastolic volumes for 6 dogs are illustrated in figure 3 and summarized in table 1. The heart rates are given for each animal; they showed wide variation during the observations in dogs 2 and 4. No major change occurred in blood pressure or in the pattern of respiration during these observations. No additional experimental procedures were undertaken during the period of these observations, nor was any anesthetic agent administered. No attempt was made to relate the time of injection to the respiratory cycle. The variability shown for stroke volume is satisfactorily small for each animal. The variability in end-diastolic vol-
Table 1

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight, Kg.</th>
<th>Observations</th>
<th>EDV* (ml)</th>
<th>SV (ml)</th>
<th>ESV (ml)</th>
<th>Average k</th>
<th>Heart rate, beats/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.5</td>
<td>10</td>
<td>21.1</td>
<td>10.9</td>
<td>10.2</td>
<td>.48</td>
<td>120</td>
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<td></td>
<td></td>
<td>(18.5-24.8)</td>
<td>(10.6-11.8)</td>
<td>(8.2-13.4)</td>
<td>(41.5-54)</td>
<td>(175-210)</td>
</tr>
<tr>
<td>2</td>
<td>15.6</td>
<td>6</td>
<td>31.1</td>
<td>14.1</td>
<td>16.6</td>
<td>.53</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(21.1-38.0)</td>
<td>(10.3-19.5)</td>
<td>(11.8-16.5)</td>
<td>(50.56)</td>
<td>(105-165)</td>
</tr>
<tr>
<td>3</td>
<td>18.0</td>
<td>5</td>
<td>44.9</td>
<td>22</td>
<td>22.9</td>
<td>.51</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(40.3-55.0)</td>
<td>(18.9-26.0)</td>
<td>(20.1-29.0)</td>
<td>(47.3-53)</td>
<td>(110-132)</td>
</tr>
<tr>
<td>4</td>
<td>18.0</td>
<td>8</td>
<td>55.6</td>
<td>19.9</td>
<td>35.6</td>
<td>.64</td>
<td>136</td>
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<td></td>
<td>(47.7-64.6)</td>
<td>(15.8-25.2)</td>
<td>(27.1-47.8)</td>
<td>(56.75)</td>
<td>(95-175)</td>
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<td>20.0</td>
<td>6</td>
<td>92.1</td>
<td>45.3</td>
<td>44.2</td>
<td>.49</td>
<td>99</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>(71.4-106)</td>
<td>(55.0-59.5)</td>
<td>(30.4-55.4)</td>
<td>(44.55)</td>
<td>(95-102)</td>
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<td>24.0</td>
<td>3</td>
<td>88.2</td>
<td>30.0</td>
<td>57.4</td>
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<td>79</td>
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<td></td>
<td></td>
<td>(72.6-100)</td>
<td>(27.6-35)</td>
<td>(45.0-70.0)</td>
<td>(.63-70)</td>
<td>(73-83)</td>
</tr>
</tbody>
</table>

*EDV = end-diastolic volume, ESV = end-systolic volume, SV = stroke volume in milliliters, and k = average of the ratios of successive concentration values (equation 4).

Effect of Duration and Timing of Injection

In all instances, relatively small volumes (0.8 to 1.6 ml.) were used. Differing durations of injection varying from 0.05 to 0.25 second had no effect on the calculation. In one experiment with the duration constant at 0.2 second the delay circuit was varied to commence injection approximately 0.1, 0.2, 0.3 and 0.4 second following the R wave. Two or 3 curves were recorded for each interval. Areas and k values were not affected by the delay. However, for the first 2 settings, dye concentrations of considerable and small magnitudes respectively were detected during the corresponding systole that triggered the injection. For such injections, then, calculations of EDV by the formula \( \frac{Q}{C_0} \) would be invalid.

An extrasystole was frequently caused by the injection of dye into the ventricle. At slower heart rates it could be avoided by commencing the injection late in systole, but under this circumstance the first peak in concentration was of less magnitude than the second. An extrasystole associated with injection rarely produced a recognizable effect on the down slope of the curve (fig. 2). However, an extrasystole or a significant spontaneous variation in rhythm during inscription of the down slope markedly affected the shape of the curve, its down slope, and the calculated values for ventricular volume. Such curves were not included in the present analysis.

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Heart rate, end-diastolic volume, end-systolic volume and stroke volume over a 30-minute period in dog 1. This animal was the most stable of the group studied and showed the least variability in volume calculations. Note the relatively small ESV, although in this animal the heart rate was above that considered optimum for recording of accurate end-diastolic concentrations using a catheter-densitometer system.

Two-Site Aortic Sampling

Two catheters were positioned in the ascending aorta and the blood-dye mixture drawn simultaneously through both at equal velocity. The dynamic responses of these systems were apparently identical. To test the accuracy of calibration and measurement, the areas subtending simultaneously recorded curves were compared. In 17 observations on 4 dogs with right ventricular injections, only 2 points showed disagreement greater than 5 per cent, indicating such errors to be minimal. For left ventricular injections, however, 6 of 16 observations showed variation in excess of 5 per cent but in only 1 was this discrepancy great (40 per cent). Since the added variability is not associated with instrumentation, these observations suggested the possibility of errors in the estimation of stroke volume associated with sampling in a location immediately downstream to the injection chamber. The finding of an even greater variability between dilution curves recorded at the aortic root and the femoral artery following left ventricular injection and between those recorded at the pulmonary artery and the aortic root following right ventricular injection lends weight to this possibility.

The position of the sampling catheter could, however, greatly affect the contour of such curves, as illustrated in figure 5, lower curve. The first change in aortic concentration of dye is not clearly detected at the more distal sampling site. This peak of concentration initially and briefly falls to a low level, followed by a rapid return and stabilization at end diastole at a value intermediate between that of the previous end diastole and early systole. Since the interval between end-diastolic concentration and the minimal value reached during this "overshoot" is 0.12 to 0.20 second, the magnitude of this change will be incompletely recorded by the catheter-densitometer system as shown in figure 1. The change so recorded may be at best 70 to 80...
Table 2

<table>
<thead>
<tr>
<th>Dog</th>
<th>Angiogram</th>
<th>Mitral regurgitation*</th>
<th>Beats to empty</th>
<th>Ventricular nonmixing*</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>4</td>
<td>1</td>
<td></td>
<td>13</td>
<td>2</td>
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<tr>
<td>2</td>
<td>1</td>
<td></td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td></td>
<td>7</td>
<td>2+</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td></td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td></td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

*Tr. = just detectable, 1 = present, and 2 = severe.

In dog 5, the tip of the injecting catheter lay just within the mitral valve.

No medium identifiable; in dog 4, medium tended to accumulate toward the apex, mixing slowly with the remainder of the ventricular contents.

Per cent of the true response, but real concentration values reaching or approaching zero for the first 0.05 second of systolic ejection are certainly possible. This type of concentration change was detected only when the sampling catheters lay just above the aortic valve and was most frequently seen at heart rates of less than 120 beats per minute. Changes in concentration of such a nature were identified in 16 per cent of all curves obtained for the data included in figure 3 and for other studies totaling approximately 200 curves. These findings demonstrate that after the injection of dye is complete the first portion of blood to leave the heart during each successive systole is apparently less dyed than that ejected later in the same systole. Mixing occurs in the aorta over such a short distance that this effect is not detected 1 to 2 cm. distal to the aortic valve.

Cineangiograms

Injection of 70 per cent solution of acetrizoate (6 to 8 ml. in 1.6 to 2.3 seconds) was frequently accompanied by 1 or more abnormal ventricular contractions, usually a ventricular extrasystole. Following this, ventricular contractions occurred in an apparently regular and normal manner. Simultaneous indicator-dilution curves were recorded from the aortic root, both with indicator dye mixed with the radiographic medium and later with this medium alone. Compared to injections of indicator dye alone, the curves following injection of both medium and dye showed a substantially greater deflection for the same quantity of dye. Further, when the medium alone was injected, a dilution curve was inscribed owing to an increased absorption of light in the spectral region of 810 mp, associated with the addition of the medium to the blood. Since quantitation of such curves presents many problems of complexity, these dilution curves were not analyzed in detail but the cineangiograms were projected and inspected simply for evidence of (1) mitral regurgitation, (2) the number of beats required for apparently complete emptying of the left ventricle, and (3) evidence for incomplete mixing in the left ventricle and aortic root (fig. 6a and b and table 2). Figure 6a is taken from the third cineangiogram of dog 3 and was interpreted to show a trace of mitral regurgitation, with 5 beats required for virtually complete emptying of the left ventricle. Evidence for severe nonmixing of newly entering blood with the residual contents of the ventricle was readily evident in frames b-1,2 and 3 and d-2 and 3 of figure 6a. It would appear that the residual volume is displaced toward the apex by the incoming left atrial blood. This could be due, however, to the higher specific gravity of the contrast medium, with accumulation of the medium in the more dependent portion of the heart. Similar evidence for nonmixing was seen to a greater or lesser extent in every cineangiogram.

Discussion

In the present study the average values for left ventricular end-systolic volume varied from 1.0 to 2.4 ml./Kg. of body weight between animals. The variability about the mean value ranged from -34 to +35 per cent within animals, but such wide variation was unusual.
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The average values are of the same general order of magnitude as reported by Holt. They are also not dissimilar to the average values of 2.1 to 3.3 ml./Kg. reported by Chapman and associates using a cineangiographic technic in the anesthetized dog. The values reported by Gribbe and co-workers, who also used cineangiography, are much smaller since the maximal end-systolic volume shown in those studies on dogs weighing 8 to 15 Kg. was approximately 10 ml.

Without question, catheter-detector systems such as those used in this study cause distortion of the dilution curve with the possibility of an overestimate of k. However, for changes occurring at a frequency of less than 120 per minute, 90 to 95 per cent of the true concentration at the end of diastole will be recorded. Indeed with such systems the presence of the very important lower concentration occurring during the phase of maximal ventricular ejection can readily be detected. Figures 2 and 5 are characterized by well-defined end-diastolic concentrations which appear to remain relatively constant for 0.2 to 0.3 second. Such curves permit no less accurate measurement than those obtained by the electrical conductivity method, which at times suffer from systolic artifact. The catheter-densitometer curves obtained by us appear not to be dynamically inferior to the published thermodilution curves of Goodyer and co-workers. It is of interest that of our values for end-diastolic ventricular volumes, all of which are within the range obtained by Holt, the smallest were obtained in the animal with the fastest heart rate (dog 1) in which the greatest errors on the basis of instrumentation might be expected.

Two sources of error exist in determinations of ventricular volumes by the dilution technic. Although the errors in the estimation of stroke volume are not under particular consideration in this paper, considerable variability was found between the areas subtended by dilution curves recorded simultaneously from the pulmonary artery and the aortic root following injection of indicator into the right ventricle for similar determinations relative to

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that chamber. The total area under the curve for the former recording site was frequently smaller and never larger than for the latter. This unexpected finding could be explained by nonuniformity of the blood-dye mixture leaving the right ventricle associated with a disparity between the sampling rate (constant) and the rate of blood flow past the sampling site (variable). The consequences of such interrelations on calculations of blood flow based on the Fick principle have been defined by Visscher and Johnson14 and by Stow.15 Likewise in determinations using simultaneous aortic-root and femoral-artery sampling, a smaller but definite variability was encountered, which appeared to exceed that associated with instrumentation. Errors in such determinations of stroke volume will magnify errors in the calculation of end-diastolic volumes since the factor (1-k) is always less than unity.

The purpose of the present report is, however, to draw attention to the occurrence of nonmixing of blood entering from the left atrium with the residual volume of the ventricle. It is not primarily concerned with the initial mixing of the injected material. The dilution curve illustrated in figure 4 shows excellent evidence for this phenomenon, and can be interpreted to indicate that the freshly entering blood mixes incompletely with the contents of the left ventricle. These observations are complemented by the studies using cineangiography (fig. 5) which by themselves could be criticized on the basis of the higher specific gravity of the opaque medium in comparison to the blood into which it is injected. This criticism, however, does not apply to the indicator dye which is probably initially distributed within the left ventricle and associates rapidly with protein molecules.

The following experiment in a 20-Kg. dog, for which we previously had no clear explanation, supports this thesis. Dilution curves were recorded from the aortic root following injection into the left ventricle. The catheter was then withdrawn to the left atrium and its tip positioned just in the orifice of a pulmonary vein, where a second injection was made. At a later time the catheter was again positioned in the left ventricle and the comparison repeated. Stroke volumes of 22 and 24 ml. were calculated from the curves recorded following left ventricular injection and of 22 and 23 ml. following left atrial injection. However, the k values for the ventricle of 0.73 and 0.69 considerably exceeded those of 0.64 and 0.60 for the left atrium, giving "end-systolic volumes" of 57 and 53 ml. for the ventricle alone compared with 39 and 39 ml. for the atrium and the ventricle. Such numerical calculations for the combined chambers are presented only to indicate a steeper decline of concentration and a faster transit time than for the left ventricle alone. If the volume of the atrium is less than the stroke volume of the left ventricle, then the former chamber acts as a channel from the pulmonary veins to the left ventricle and all of the indicator dye would be rapidly washed into the latter chamber. Should this blood-dye mixture then fail to mix with the residual volume of the left ventricle, a faster washout from the 2-chamber system than from the ventricle alone could occur.

As previously stated, evidence for left ventricular nonmixing was obtained in 16 per cent of the dilution curves. However, differences of as little as 1 cm. in the position of the tip of the sampling catheter in the aortic root abolished the occurrence of such fluctuations in the recorded indicator-concentration curve. Thus it is possible that the incidence of nonmixing was very much higher than indicated by the aforementioned figure.

If serious nonmixing between the left atrial blood and the end-systolic volume of the ventricle exists, then calculations based upon a simple washout system no longer give valid results, since the degree of mixing between the newly entering blood and the end-systolic volume is unknown and probably variable. Accurate formulation may thus be impossible and the values so derived must give rise to the calculation of excessive volumes. This is the reverse of the situation described by Newman and co-workers16 in which failure of the entering dye to mix completely with the cham-
ber contents results in a falsely low estimation of chamber volume. In the present situation, it is the failure of the entering undyed blood to mix with and clear the residual dye from the left ventricle which causes an erroneously high value for chamber volume. Neither the characteristics of the detecting instruments nor the nature of the injected material is concerned with this process. The magnitude of such errors would increase for larger as compared to smaller true volumes. If the end-systolic volume is zero, there is no mixing problem and no error. When the end-systolic volume exceeds 50 per cent of end-diastolic volume, then the possibility of serious non-mixing with further overestimation of end-diastolic volume must be considered. Such a possibility is a likely cause for the wide variability between and within animals found in the present study and in the literature.

Summary

1. In the anesthetized dog, end-diastolic, end-systolic and stroke volumes for the left ventricle have been calculated according to the method of Holt from dilution curves recorded at the aortic root. For heart rates of less than 120 beats per minute, the catheter-densitometer systems used gave 90 to 95 per cent of the true concentration in the aortic root at the end of diastole.

2. Average volumes of 2.96, 1.63 and 1.33 ml./Kg. of body weight were calculated for each of these parameters. The interindividual and intraindividual variations about these means amounted to ± 30 to 50 per cent.

3. In approximately 15 per cent of the dilution curves the concentration of dye in the aortic root during early systole (phase of maximal ejection) showed a transient, markedly low value compared with that of the end of diastole. This can be explained only on the basis of the preferential ejection from the heart in early systole of the undyed, newly entering blood from the left atrium, which had mixed poorly with the residual volume of the ventricle. Qualitative inspection of cineangiograms confirmed this impression and demonstrated that a residuum of contrast medium failed to mix with the incoming blood from the left atrium.

4. In addition, there is evidence for the possibility of overestimation of stroke volumes from dilution curves recorded immediately proximal to the injection chamber.

5. Incomplete mixing of the left artial blood with the residual volume of the ventricle precludes accurate quantitation of the changes of volume in the ventricle by the simple formulas proposed by previous authors. This phenomenon may serve to explain the considerable variability that is found in the present study and in the literature.

Acknowledgment

We are indebted to Dr. Kenneth H. McLean and Mr. W. Arthur Meeker for assistance with the experiments, and to Mr. Donald Twentyman (student fellow of the Minnesota Heart Association) for assistance in the analysis of the data.
sinistro-atrial con le volumine residue del ventriculo rende impossibile la accurate quantitation del alterationes de volumine in le ventriculo per le simple formulas proponite per previo autore. Iste phena-omeno servi forse a explicar le considerabile grados de variabilitate quo esseva incontrate in le presente studio si ben conio in alteres jam in le litterature.

References
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